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3.3.1 INTRODUCTION

All animals are continually exposed to micro-organisms present in the atmosphere, in the food and in the environment. The external surfaces of the animal, the alimentary tract, and in higher animals the eyes, nose and urinogenital regions offer a wide range of potential colonization sites, but they do not all offer the correct conditions for microbial survival and replication. Indeed, a variety of physical, chemical and ecological conditions and host defences may mitigate against the survival of the micro-organisms.

Exposure to environmental micro-organisms commences during birth in viviparous animals, or after hatching from the egg in birds, reptiles and most invertebrates. Successive invasions of micro-organisms occur in each habitat offered on or in the animal. The newly arrived micro-organisms integrate with, or displace, previously successful species until eventually, under stable environmental conditions, a climax population is established in each of the habitats. Species which occur in this climax population in all members of the animal community, and which in all probability were present during the evolution of the animal, are defined as indigenous or autochthonous species; other species which may occur in the same habitat, but do not normally establish themselves there in the absence of perturbations of the system, are termed allochthonous species [136]. Many allochthonous species are present in most animal habitats at all times, but some autochthonous species are unique to a single type of animal habitat, and some may be restricted to a single species of animal.

The indigenous organisms associated with external surfaces of the animal often apparently offer little benefit to the host, although they may offer some protection against pathogens. Conversely, indigenous micro-organisms within the alimentary tract have been exploited by many herbivorous animals for the digestion of plant structural carbohydrates and other beneficial functions such as the utilization of non-protein nitrogen, detoxication of dietary components and provision of vitamins. With the possible exception of a few invertebrates, no animals can digest cellulose, hemicellulose or pectin without the aid of these symbiotic micro-organisms. Because the rate of enzymic hydrolysis of cellulose and hemicellulose is slow, the rate of passage of plant tissues through the alimentary tract of herbivorous mammals and some insects (for example, wood-eating termites) is slowed by the provision of relatively large chambers in which the microbial hydrolysis of plant polysaccharides occurs. Such chambers are either pregastric complex stomachs as found in ruminants, kangaroos, colobid monkeys, camels, llamas and some whales, or are post-gastric chambers, such as the large intestine and caecum occurring in primates, pigs, horses, many rodents, birds and some reptiles. Many mammals with a pregastric chamber also possess a well-developed large intestine and caecum where additional fermentation of dietary components occurs.

One of the most surprising aspects of the animal–

micro-organism system is the enormous numbers of micro-organisms that are involved. It has been calculated [81] that the human being may be colonized by as many as 10^{14} indigenous microbial cells and that, of the total human–micro-organism community, only 10% of the cells were human [132]. In the sheep rumen, viable bacterial populations as high as 2×10^{10} cells g^{-1} of digesta are often recorded. If each organism were only 1 μm long, those present in a rumen with a fluid volume of 5 litres would stretch, end to end, 2.5 times round the earth.

The types of habitat provided by the animal for micro-organisms vary from fully aerobic sites, such as those found on external surfaces, to highly anaerobic sites of low redox potential in the alimentary tract. Sites of intermediate redox potential occur in the oral cavity and lower reproductive tract. In external environments the habitat may be relatively dry with a limited nutrient supply; cells are often removed by abrasion and sloughing of the external layer of skin or cuticle. Internal environments are usually moist, with abundant nutrients. The temperature of the habitat varies from ambient on the external surfaces of invertebrates and cold-blooded animals to slightly above core body temperature in the gastrointestinal tract of mammals.

Many of the bacteria in a wide variety of aqueous systems, including the gastrointestinal tract, grow in microcolonies attached to surfaces surrounded by a glycocalyx through which molecules and small particles pass [46, 47]. The glycocalyx consists principally of exopolysaccharide which binds to both the bacterial surface and the substratum; it probably protects the bacteria from bacteriophage or colicin attack, traps nutrients by its ion exchange capacity and minimizes loss by abrasion. The surface of some animal cells lining the alimentary tract is enclosed in a polysaccharide glycocalyx [37] presenting a surface similar to that of the bacterial glycocalyx [127]; this surface can supply nutrients to adherent bacteria in the form of polysaccharides and glycoproteins. In the rat, the mucus layer lining the mid-colon may also prevent adhesion of luminal bacteria to the colon epithelium [19].

Some autochthonous micro-organisms perform only a limited range of functions in their natural environment and it is easy to allocate a niche to these species. In contrast, others have a broader range of functions. For example, micro-organisms with some flexibility of substrate utilization may change their functions according to the carbon sources present. Thus no specific niche may be allocated to these species. More intimate associations of micro-organisms with animals occur where the organisms are enclosed within the animal cells in direct contact with the cytoplasm of the host. These endosymbionts are usually found in particular organs or specific anatomical regions such as, for example, those associated with the gastrointestinal tract of some insects.

The role of micro-organisms in many animal–microbe systems is incompletely understood. Much research has been conducted on microbial communities in ruminants, man and invertebrate pest species, but many of the principles operating undoubtedly apply in others.

3.3.2 MICROBIOLOGY OF EXTERNAL SURFACES

The skin and lower genital tract of man

The skin is sterile before birth, but during birth it is contaminated by the vaginal flora. In normal healthy individuals, the skin is highly resistant to invasion by many species of bacteria. The keratinized epithelium provides a physical barrier, and its dryness, low pH (5.5), the production of antibacterial compounds such as long chain unsaturated fatty acids in sebaceous secretions of glands, and interference between bacterial populations all exert some control over which species dominate the population of the skin [139, 163].

Bacteria inhabiting the skin belong either to a resident population or to a transient population. The transient population represents species from the environment or species such as *Staphylococcus aureus* more commonly found in the nose which in some individuals periodically occur in large numbers on the skin [99]. The resident flora consists of species normally found on the skin, but the species composition varies considerably with the anatomical site. The major anatomical regions can be divided into three groups: the exposed surfaces (face, neck, hands); the moister regions (armpits, perineum, toewebs) and the remainder (trunk, the remainder of arms and legs). Higher bacterial population densities occur on the exposed surfaces, where populations are dominated by corynebacteria, micrococci and sometimes *Staph. epidermitidis*. In the moister regions, Gram-negative bacteria including *Pseudomonas* spp. are more common but rarely dominant, except in some pathological conditions.

Counts of bacteria present on the skin vary according to the methods used; surface samples indicate a bacterial population density of up to 8×10^4 cm^{-2} for the armpit and 2.3×10^4 cm^{-2} on the forearm. However, skin from cadavers which was homogenized and treated with Triton X-100 yielded population densities of 9.2×10^5 cm^{-2} and 1.3×10^6 cm^{-2} for the armpit and scalp, respectively [160]. Electron microscopy has revealed bacterial colonies both on the skin surface and in the stratum corneum [90]. The different values obtained by the two methods is explained by this observation. Undoubtedly the highest bacterial populations occur on the head, armpit, groin, perineum, hands and feet; numbers greatly increase after hydration of the skin by washing. For this reason bacterial colony counts from female skin are generally higher than counts from male skin, because of more frequent washing.

The eyes are well protected from bacterial invasion by lachrymal secretions which wash them free of micro-organisms; the secretions drain through tear ducts to the nose. These secretions contain lysozyme, which kills susceptible bacterial cells and helps prevent bacterial invasion of the eye socket.

Although much is known of pathogenic microbial species inhabiting the female lower genital tract, only a little is known of the normal microbial flora [67]. One microbial habitat is the walls of the vagina; its inner surface consists of stratified epithelium lubricated by secretions of the cervix and is clearly favourable for the growth of anaerobes. Glycogen in the vaginal mucosa is hydrolysed (probably by enzymes in the epithelial cells) and provides substrates which are fermented by micro-organisms. Acids are produced which maintain the vaginal pH at 4–5 in sexually mature women. The posterior fornix of the vagina is therefore inhabited by species tolerating a low pH including streptococci, yeasts (particularly *Candida albicans*), anaerobic lactobacilli, coliforms and mycoplasmas, many of which are of potential harm to both the newborn baby and the mother.

Another habitat, the skin of the labia majora, contains sebaceous glands which secrete bacterial inhibitors and sweat glands which provide ammonia and other substrates for microbial growth. The microbial population here is dominated by *Corynebacteria, Staph. epidermitidis* (common skin bacteria), lactobacilli, faecal streptococci and micrococci.

Microbiology of invertebrate integuments

The micro-organisms present on invertebrate integuments have a wide spectrum of relationships with the host, from acting as pathogens, through the benign or commensal relationships of most invertebrate–microbial interactions, to the close mutualistic symbioses illustrated by the carriage of fungal inocula in special integument structures by ambrosia beetles. In all these cases the species complement and abundance of the epibiota are affected by a combination of integument characteristics, the habitat in which the animal lives and the associated environmental conditions. These variables operate in a hierarchical series: the gross physicochemical environmental conditions determine the form, abundance and identity of the micro-organisms potentially available as colonists; the occurrence of an animal in a habitat associated with particular microclimatic conditions favours closer contact with a more specific component of the microbial community and the integument characteristics of the animal then operate at the finest level of selection.

The behaviour of the animal is an important modifying factor and aggregation, dispersal, feeding activities and even grooming influence the species complement and abundance of attached micro-organisms.

These factors do not have equal weighting and in particular circumstances one or another may be dominant, but this holistic concept serves to emphasize that, in searching for ultimate factors to account for particular ecological phenomena, it is easy to introduce artefacts or draw false conclusions as a consequence of isolating the animal from its natural environment. Unfortunately, in many studies this supporting detail is incomplete. The ecology of attached micro-organisms will therefore have to be assembled from discrete details starting with the nature of the surface layers — integuments — of invertebrates.

BODY SURFACE CHARACTERISTICS

The basic structure of the invertebrate integument is elaborated in different ways in different groups of invertebrates but in terms of microbial attachment there appears to be a fundamental division between soft-bodied animals, where the superficial layers of the integument are living structures, and the arthropods with exoskeletons where the integument surface is more or less inert.

In the coelenterates (including the stony corals), free-living annelids and most groups of molluscs, secretions of mucus and protein play a large part in protecting the epidermis. The mucopolysaccharides are rapidly degraded by micro-organisms but the dynamic processes of more or less continuous secretion reduce microbial attachment. In addition bacteriolytic agents have been identified in earthworm mucus [151] and may be found in the epidermal secretions of other groups. The echinoderms are coelomates and have an internal calcareous skeleton, but the body surface is similar to that of the corals in having a sheet of ciliated epidermal cells covering the test. The cilia clean the surface of the animal and prevent bacterial or algal growths. A powerful bacteriocide is also present in the coelomic fluid, which may influence bacterial attachment.

The opposite extreme is found in bivalve and gastropod molluscs where an inert calcareous shell encloses the body. Algal growths may be observed on the shells of sessile intertidal mussels and limpets but the majority of gastropods appear free of epibionts. This may be due to the inhibition of microbial colonization by the periostracum, a thin horny layer of conchiolin covering the shell; the abrasion of this layer may facilitate attachment.

The procuticle in arthropods is soft and flexible when first secreted by the epidermis but becomes hardened with tanned proteins. In terrestrial arthropods, and particularly in insects, layers of wax are deposited on the surface of the tanned procuticle, waterproofing the integument and providing a hydrophobic surface layer. The wax may be further protected by a hard layer of tanned protein. The detailed structure and functioning of arthropod and insect cuticles varies widely between groups and is reviewed in [80] and [98].

MECHANISMS OF MICROBIAL ATTACHMENT

There is much information on micro-organisms isolated from the external surfaces of invertebrates, considered in the following section, but the mechanisms of attachment have not generally been investigated.

Many fungi produce sticky spores which adhere to the integument [70]; this is the principal means of dispersal in some species such as *Ceratocystis* spp., which include Dutch elm disease. On the other hand it has been shown [129] that the strongly hydrophobic spores of *Streptomyces griseus* are attracted to arthropod cuticles and hence this could be true of other micro-organisms.

Studies of the mechanism of bacterial adherence to mammalian tissue culture cells have shown that the degree of hydrophobicity of the bacterial surface is correlated with the ability of the organisms to adhere to the cells. The hydrophobic molecules allow the bacteria to approach the negatively charged epithelial cell and thereby enable binding molecules on each of the cell surfaces to interact and form specific bonds [106].

Little work has been carried out on the specificity of microbial attachment to invertebrate surfaces except for pathogenic fungi, where the stimulus for research has been the development of biological control measures against insect nematode pests. Entomopathogenic fungi normally invade through the surface integument and so a spore must remain in contact with the cuticle long enough for germination and invasion to take place. For this purpose *Entomophthora* spp. produce sticky spores, which germinate and penetrate the thin cuticles of flies, mosquitoes and aphids [84] at any point. In larger insects, penetration of *Entomophthora* is through the non-sclerotized membranes between the segments, in physically protected, humid sites. The setae and setal sockets are covered by a cuticle which is much thinner than that of the integument and some Laboubeniales germinate and penetrate at these sites [149]. If the thallus cannot penetrate at the point of germination, a haustorium is extended to a more susceptible area.

Some entomopathogenic fungi, however, appear to have highly specific attachment sites. An example is *Coelomomyces psorophorae* where the distribution of cysts on the integument of mosquito larvae indicates that sites are identified by differences in cuticle texture [35]. The final product of attachment, host invasion, may be a result of enzymatic degradation of the cuticle but mechanical penetration by haustoria appears to be the main mode of entry through the host integument.

More than 150 species of fungi are known to attack nematodes or their eggs [8] and these species can be classified into predatory and endoparasitic forms. The predatory fungi capture nematodes by adhesive devices (hyphae, knobs, branches, nets) or non-adhesive snares and hyphal loops. After capture the nematode cuticle is penetrated and the fungal hypha enters the body.

The endoparasitic fungi have no hyphal development outside the body of the host and infection is generally by adhesive spores. In *Meria coriospora* the spores are teardrop shaped with a small adhesive bud at the distal end. They can attack at any point on the surface of the nematode but do so mostly in the region of the mouth and on sensor organs. Nordbring-Hertz and colleagues have carried out a series of elegant experiments, reviewed in [101], demonstrating that site recognition by *Meria*, and nematode capture by some trapping species with adhesive knobs and hyphal networks, is initiated by a lectin–carbohydrate interaction between the nematode and fungal surfaces.

MICRO-ORGANISMS ASSOCIATED WITH INVERTEBRATE INTEGUMENTS

Most hard-bodied invertebrates have an extensive surface microbiota which broadly reflects the species complement of fungi and bacteria characteristic of the habitat where the animals are found. Mobile invertebrates will therefore act as passive carriage and dispersal agents for microorganisms. In other cases, almost exclusively involving fungi, dispersal is primarily effected by the 'deliberate' or active carriage of inocula by insect vectors. In passive dispersal propagules may be found on any part of the integument, but in active carriage propagules may be confined to specialized morphological structures. Propagules may also be dispersed actively or passively in animal guts.

It would seem reasonable to assume that the species complement of bacteria associated with invertebrate integuments would reflect the animal's habitat. The integument of the marine wood-boring isopod *Limnoria lignorum* was dominated by *Pseudomonas* and *Vibrio* species, which are common in the water, as well as *Aeromonas hydrophila*, which was associated with the wood from which the animals were collected, but was not isolated from water [21]. Similarly *Pseudomonas* and *Bacillus* species might be expected to be prevalent on the exoskeleton of terrestrial arthropods but, paradoxically, only *Streptomyces* (Actinomycetes) were isolated from the exoskeleton of woodland millipedes [145]. No Eubacteria were recorded on the integument, even though Enterobacteriaceae were common in the faeces. These results imply that the attachment of *Streptomyces* spores is more specific than the electrostatic mechanism previously suggested [129]. Differential survival of the bacterial inocula would not seem to be implicated, given the moist conditions in which the millipedes live. In contrast, various species of flies have been found to carry a wide range of human pathogenic bacteria, including *Shigella dysenteriae* (bacterial dysentery), *Salmonella typhi* (typhoid) and virulent strains of *Escherichia coli*. The relatively few bacteria on a fly's feet, picked up from food or faeces, soon dry up and are killed. Gut carriage and transmission by faeces is a far more important mechanism of passive dispersal of pathogens.

The fungi associated with soil invertebrates reflect most of the common saprophyte genera (*Mortierella*, *Cladonia*, *Penicillium*, *Aspergillus*, etc.), which sporulate abundantly in dead organic matter [15, 117]. Spores of invertebrate parasites (*Beauvaria* and *Paecilomyces*) and plant pathogens (e.g. *Fusarium*) are also found. Most studies on Collembola and mites suggest that these animals carry inocula of about 20 species [153] but the number of genera appears to be related to body surface area; microarthropods had an average of 0.5 species per individual while beetles and worms had one or two species [117]. A total of 120 taxa of fungi were isolated from tracts and whole-body squashes (which include fungi in gut contents) of species of woodland collembola. The species present on the integument were generally similar to those present in the soil and litter horizons from which the animals were extracted. Two exceptions were the parasite *Beauvaria bassiana*, which was more abundant on collembola than in soil, and *Trichoderma* species which were never isolated from the animals. Soil invertebrates may therefore be important passive dispersal agents for fungal spores in soil and litter systems. The carriage of spores may even be an essential dispersal mechanism for many wood-rotting basidiomycetes which form sporophores on the underside of branches and logs on the forest floor, where air currents are unlikely to be sufficient for aerial dispersal [147].

Above ground, wind and rain are generally considered to be more important for the dispersal of fungi. However, the physiological characteristics of *Ceratocystis* spores make it unlikely that these species are air-dispersed [50]. Transmission takes place when *Scolytus* spp. beetles disperse from their breeding material in spring and fly to the tops of healthy elm trees to feed on young sappy bark in twig crotches. Some of the wounds are contaminated with spores of *C. ulmi* and the fungus spreads through the xylem vessels of the tree, producing the characteristic wilt symptons. Once the tree is dead or dying, the female beetle excavates a breeding gallery in the bark where the larvae develop, surrounded by fungus in and around the chambers. Pupation occurs in the autumn and the beetles emerge bearing loads of 250–2500 viable spores for *Scolytus scolytus* with a range from 1 to 20 000 spores [156]. Although three spore types are produced by the fungus, the spore load on the beetles is dominated by one type, conidiospores, because of the synchrony between beetle emergence and the phenological characteristics of the fungus (see Fig. 3.3.1).

Active (epizootic) dispersal of fungal propagules carried in body pouches (mycangia or mycetangia) is characteristic of a number of insect groups, notably the ambrosia beetles, woodwasps and some termites, which exploit living or dead wood as a food resource. The winged females of leaf-cutting ants, which maintain fungal monocultures in fungus gardens through a series of physical and biochemical controls [143], also transport pellets of hyphae from the garden in a mycangium located below the mouth [157].

There are more than 1500 species of ambrosia beetles (Scolytidae), mostly tropical species in the tribe Xyloborini and members of the genus *Xyloborus*. Each species is associated with one or more species of Ascomycetes or Deuteromycetes with *Fusarium* the major genus. Many

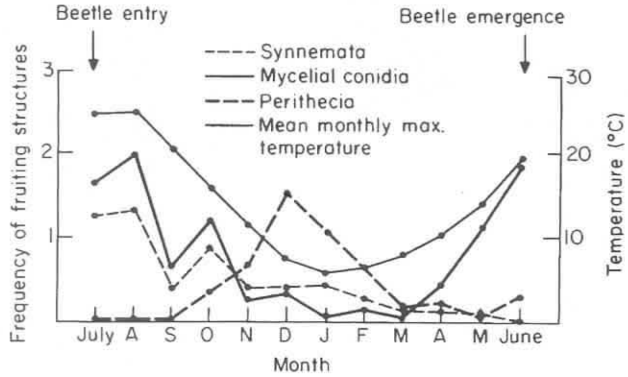

Fig. 3.3.1. Changes in the frequency of the three spore stages of *Ceratocystis ulmi* in beetle breeding galleries between beetle entry (left) and beetle emergence (right). (Copyright Forestry Commission. After Webber & Brazier [156].)

species attack unhealthy or newly felled logs, and are often restricted to a single family of trees. Ambrosia beetles have been studied for over 150 years because of their economic importance and reviews have been published on this group and their fungal associations [77, 102, 103].

The female beetles carry the fungal propagules in small pouches (mycangia) on the exoskeleton; these may be membranous pouches at the base of the mandibles, or sclerotized pouches at the base of the elytra or between the thoracic segments. The fungus is carried as a yeast-like form which multiplies while in the mycangium. When the beetle starts boring to construct the brood galleries, glands associated with the mycangium produce a copious oily secretion which flushes out the fungal inoculum. This inoculum germinates on the tunnel walls to produce a new growth of ambrosia: a tightly packed mass of conidiophores which form the adult and larval food.

The ambrosia beetles have co-evolved with the fungal symbionts to the extent that initiation of reproduction in the female is dependent upon the high concentrations of lysine, methionine, arginine and histidine synthesized by the fungus. It has also been demonstrated that the nutritional basis of the symbiosis for the growth of the larvae of one species is the provision of ergosterol by the fungus [77].

Syricid woodwasps infest a wide range of hard and soft woods in the northern hemisphere. The adult female drills through the bark of the tree into the xylem where the eggs are deposited together with fungal spores from a pair of pouches located at the base of the ovipositor. The larvae also have paired mycangia on the first abdominal segment where spores are held in a dormant condition embedded in wax platelets. The larval integument is shed at pupation but the wax platelets in the exuvium are shattered by the movements of the emerging adult. The spores then germinate and the fungus grows rapidly to invade and inoculate the mycangium of the female before she emerges from the tree [85]. Fungi associated with the woodwasp, including the basidiomycetes *Stereum sanguineolentum*, *Stereum chailletii*, *Amylostereum areolata* and *Daedalea unicolor*, appear to play a complex role in the ecology of the insect in that they may facilitate host invasion by weakening the tree, as well as having nutritional significance for larval development.

LUMINOUS BACTERIA

Bioluminescence on land is found in fungi, bacteria and some insect groups, notably Coleoptera (fireflies and glow-worms). Numerous records exist of bioluminescent invertebrates, but these mostly represent contamination of the integument by luminous bacteria from the moist habitats in which these animals are found. Some animals, however, are infected with pathogenic luminous bacteria by parasitic nematodes [118]. The bacteria infect the haemocoele of caterpillars and other insects when the nematode has burrowed through the gut wall. The bacteria multiply, killing the host, and the progeny of the nematodes are reinfected as they complete their life cycle in the insect's haemocoele. The functional significance of this symbiosis is unknown.

Bioluminescent bacteria have been most extensively studied in the marine environment where they occur free-living, as commensals on the integument of many animals and in specific organ-bound associations. Recent studies [11] indicate that there are two main taxonomic groups: the genus *Photobacterium*, found mainly in symbiotic association with cephalopods, tunicates and fish, and *Beneckia* (*Vibrio*), which are free-living.

The symbiotic bacteria are located in special organs in a variety of shapes and locations, often accompanied by morphological adaptations which act as lenses, reflectors or shutters to control light emission (see Fig. 3.3.2). Two fish which live in the coastal Indonesian seas, *Anomalops katoptron* and *Photoblepharon palpebratus*, have large bean-shaped luminous organs under each eye which can be covered with a membrane so that it flashes periodically [88]. Many deep-sea fish have luminous organs but

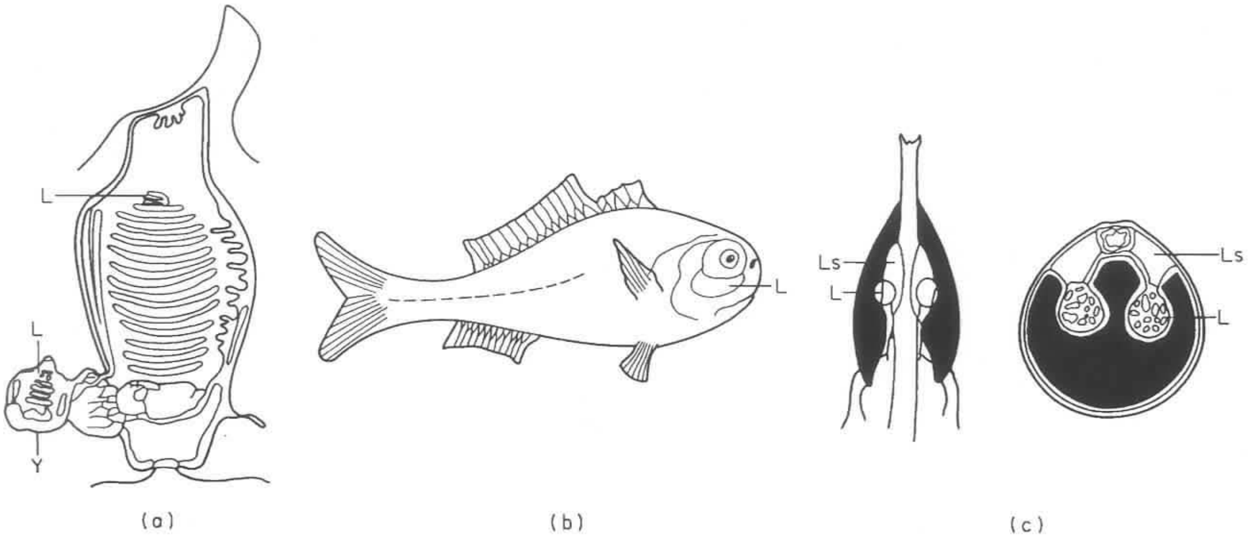

Fig. 3.3.2. Luminous organs which contain symbiotic luminous bacteria. (a) *Pyrosoma* sp. (a tunicate). An individual of a colony in side view with a luminous organ (L) above the gill-intestine. A young animal (Y), developed on the stolon, also has a luminous organ. (b) *Anomalops katoptron*. The bean-shaped luminous organ (L) is situated below the eye. (c) *Loligo edulis* (squid). A male animal with spindle-shaped lenses (Ls) and round luminous organs (L), and a cross-section through the trunk showing the luminous organs embedded in the ink sac and the lenses which flank the rectum. After Buchner [24].

in most species these are chemoluminescent and light generation does not involve bacteria. Similarly, of the 19 families of luminous squids, only two families have genera which use luminous bacteria as their source of light [60].

The mutualistic basis of the symbiosis accrues from the provision of nutrients and a protected environment for the bacteria while the light can be used to attract prey, to assist in escaping or diverting predators, and for communication [91] by the host animal.

Quantitatively, however, the most important habitats of luminous bacteria are the guts of marine animals including mussels, scallops, crabs and fish. Many marine fish carry these bacteria in their intestinal tract at population densities of 0.5 to 5 × 10^7 cells ml^{-1} of gut material. These bacteria may be important in the digestion of chitin [128].

3.3.3 VERTEBRATE GUT SYSTEMS

The vertebrate gut as a habitat

Unlike the external surfaces of animals, the alimentary tract offers many habitats highly conducive to microbial growth. A generalized diagram of the alimentary tract is shown in Fig. 3.3.3, and the relative capacities of different regions of the tract are given in Table 3.3.1. Numerous differences in the anatomy of the tract occur in the animal kingdom [9, 82], each related to the diet of the animal. Each segment of the gut provides different environmental conditions for microbial growth; these conditions frequently change in some way as the animal matures. In some sites definite successions of micro-organisms have been recognized, commencing at or soon after birth [95, 109]. Three interacting populations of micro-organisms may be found in each region of the gut: those free in the aqueous phase of the gut lumen, those attached to food particles, and those adherent to the epithelium lining the tract [36]. In some regions of the tract, such as the abomasum of cattle and the ileum of the mouse, a continuous viscous stream of mucus flows over the epithelium surface and this viscous stream contains most of the bacteria and protozoa. Despite earlier ideas, the number of micro-organisms actually attached to the epithelium in these sites is small [47]. Population densities for lumen micro-organisms and environmental conditions at different sites in the alimentary tract are given in Table 3.3.2. In addition, the structure and

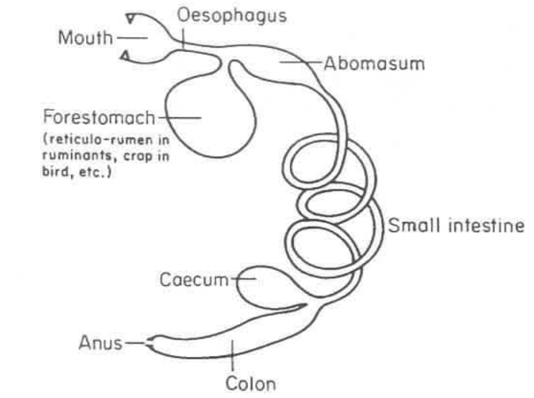

Fig. 3.3.3 Generalized diagram of the alimentary tract of animals.

Table 3.3.1. Parts of alimentary tracts of mammals, expressed as % of total tract

	Pig	Cow	Horse	Rabbit
Stomach	20	71	9	34
Small intestine	33	19	30	11
Caecum	6	3	16	49
Colon	32	8	45	6

Table 3.3.2. Microbial populations ($\log_{10}$ per g of organ contents), redox potential and pH of regions of the human alimentary tract*

	Stomach	Jejeunum	Ileum	Colon
Aerobes and facultative anaerobes	0–3	0–4	3–8	6–10
Anaerobes	0	0	2–7	8–12
Total	0–3	0–4	5–8	10–12
E_h (mV)	+150	−50	−150	−200
pH	3.0	6–7	7.5	6.7–7.3

* Condensed from [49].

physiology of sections of the tract may change with the age of the animal. This is best exemplified by the young ruminant, which does not have a fully functioning rumen until after 3–4 months.

Chewing of the food is an important first step in digestion, as the reduction in particle size increases the surface area of food available for subsequent attack by digestive enzymes. At the same time, as it is being chewed the food is mixed with saliva, which buffers the pH, generally contains some digestive enzymes, and helps to lubricate the passage of food through the oesophagus.

The mouth

The mouth of a mammal presents a variety of sites for colonization by ingested bacteria, and may be regarded as an ideal microbial incubator. Not only is the temperature maintained near core body temperature (at 35–36°C in man), but the abundant water and food provides a rich variety of microbial substrates, as well as habitats for both aerobes and anaerobes. The mouth normally harbours a complex microflora, with each species having differing growth rates, nutrient requirements and preferred sites for growth [100]. Bacterial population densities of 10^{11} (g wet wt)$^{-1}$ in plaque and 10^{9} ml^{-1} of saliva have been recorded [57].

The microbial population mostly occurs attached to the teeth, the cheeks and the tongue and in gingival crevices (see Table 3.3.3). The species composition of populations differs from site to site; for example, species occurring on the aerobic crown of the tooth differ from those of the anaerobic gingival crevices, and cheek populations differ from tongue populations. The microbial population of the saliva represents mostly attached cells which have become dislodged by abrasion or washed off by saliva. The upper limit of the microbial population is controlled by the net effect of washout by salivary flow, abrasion during eating, and washout of cells from gingival crevices by tissue fluids.

In man, the mouth of the child is quickly contaminated after birth by a variety of micro-organisms, principally streptococci, staphylococci, coliform bacteria and Gram-positive rods originating from the mother and local environment. The initial population is mostly of aerobes and facultative anaerobes, but changes occur in a more or less definite succession because of alterations in oral conditions with the age of the child. This is best demonstrated by the succession of *Streptococcus* spp. During the first 48 hours after birth, the oral mucosa does not bind streptococci but *S. salivarius* is normally present in the saliva [107]. This species is probably transferred directly from the mother [27]. After the teeth have erupted, the gingival crevices are invaded by facultative anaerobes, including *S. sanguis*, which attaches more strongly to tooth surfaces than *S. salivarius* [28]. In the gingival cre-

Table 3.3.3. Approximate proportional distribution (%) of more common bacteria on oral surfaces and in saliva*

	Gingival crevice	Coronal	Plaque	Tongue	Buccal	Dorsum	Saliva	Mucosa
Str. salivarius	<1	<1		20	11		20	
Str. mitis	8	15		8	60		20	
Str. sanguis	8	15		4	11		8	
Str. mutans	—	1		<1	<1		<1	
Gram-positive filaments	35	42		20			15	
Veillonella sp.	10	2		12	1		10	
Bacteroides oralis	5	5		4				
Bacteroides melaninogenicus	6	<1		<1	<1		<1	
Enterococci	0–10	<0.1		<0.1	<0.1		<0.1	
Others, unidentified	>27–19		>17		>29	>14		>24

* Modified from Gibbons & van Houte [57].

vices, the redox potential decreases and the habitat becomes suitable for true anaerobes such as *Bacteroides melaninogenicus*, *Haemophilus* spp. and *Spirillum* spp. After about 1 year, *Streptococcus mutans* is found. Both *S. sanguis* and *S. mutans* are cariogenic plaque species and of considerable significance in oral health. In contrast, *S. mitis* is the dominant species on the buccal mucosa.

Many oral bacteria produce extracelluar dextrans, laevans and other polymers which form a glycocalyx surrounding the cells, binding colonies of different organisms together to form dental plaque [26]. Many of the glycocalyx components can themselves be hydrolysed by mouth bacteria, but mutan (an α-1, 3-glucan) produced by *S. mutans*, a common cariogenic plaque species, is water-insoluble and less easily hydrolysed by bacteria. Mutan is probably of major significance in maintaining the structural integrity of the plaque, and acidic conditions (pH 6.0) generated in carious lesions help maintain populations of the acid-tolerant plaque species. Plaque formation is not restricted to man, but is widespread in the animal kingdom [48].

Although continually exposed to reinoculation by environmental micro-organisms, the mouth has defence mechanisms which restrict invasion and growth. These mechanisms of anatomical and physiological barriers include mucous membranes, squamous epithelium, the flow of saliva, the anatomy and chemical composition of the teeth, and microbial products inhibitory to growth, phagocytosis by macrophages and leukocytes, humoral antibodies and cell-mediated responses in gingival crevices, and salivary lysozyme of immunoglobulins [100].

Less is known of the oral microbiology of animals, but many similarities undoubtedly exist between microbial populations in warm-blooded animals and man. In ruminants, regurgitation of food results in a large number of rumen organisms entering the mouth but rumen anaerobes probably do not survive there. Populations of the filamentous bacteria *Alysiella* spp. and *Simonsiella* spp. occur on the tongue [83]; these species are tenaciously adherent, even in this highly abraded environment.

In monogastric animals, food leaving the mouth passes down the oesophagus to the stomach. In species with a pregastric fermentation chamber such as the ruminant, the food passes first into the forestomach before passing on to the omasum, abomasum and remainder of the alimentary tract.

Forestomach fermentation systems

Considerably more is known of the rumen microbial communities than other forestomach fermentation systems. The young suckling ruminant is, however, essentially a monogastric animal. Ingestion of liquids stimulates the reflex closure of a structure known as the oesophageal groove, which channels the food directly to the abomasum and bypasses the undeveloped rumen [30]. When the young animal starts to eat small amounts of forage, the oesophageal groove does not close and forage is allowed to enter the immature rumen. The rumen increases in size and, with an increased forage intake and modification of its flora, becomes functional. The rumen microbial population initially consists of facultative anaerobes, principally lactic streptococci and lactobacilli, which ferment the small quantities of milk which accidentally enter the rumen from the oesophageal groove. These organisms also decrease the redox potential, which allows a succession of increasingly oxygen-sensitive organisms to proliferate and ferment the initially small quantities of ingested forage. Inoculation is direct from the mother, by aerosol or by eating contaminated feed, and typical anaerobic rumen bacteria occur in low numbers within the first days of the animal's life. In sheep the climax population may not be reached for 3–4 months when the rumen becomes fully functional [66]; the microbial flora of the caecum, however, is functional within 15 days of birth [109].

The food is prepared for microbial metabolism by an initial chewing and mixing with saliva in the buccal cavity to form a bolus. Saliva is produced copiously and continuously, and an adult cow produces about 60–100 l day^{-1} The saliva is rich in bicarbonate, phosphate, sodium, calcium and potassium ions, contains mucoproteins and urea and usually buffers the rumen contents to pH 6.4–6.8 [66]. The bolus is swallowed and is moved down the oesophagus into the rumen. Here the plant fragments are invaded and colonized by anaerobic and facultatively anaerobic rumen micro-organisms. The invasion process is a mixture of active invasion involving chemotaxis in some species (ciliate protozoa, anaerobic chytridiomycete zoospores and motile bacteria) and adherence by non-motile bacteria. One of the first of the ciliate species to invade freshly ingested plant tissue is *Isotricha intestinalis* (see Fig. 3.3.4A), followed by the ophryoscolecid ciliates, chytridiomycete zoospores and bacteria (see Fig. 3.3.4B). *Isotricha intestinalis* is a large, highly motile species; it has been observed that 90% of the population of this species in the rumen fluid transfers to plant tissues within 5 min of their ingestion [112]; the same species also migrates to and from the rumen epithelium.

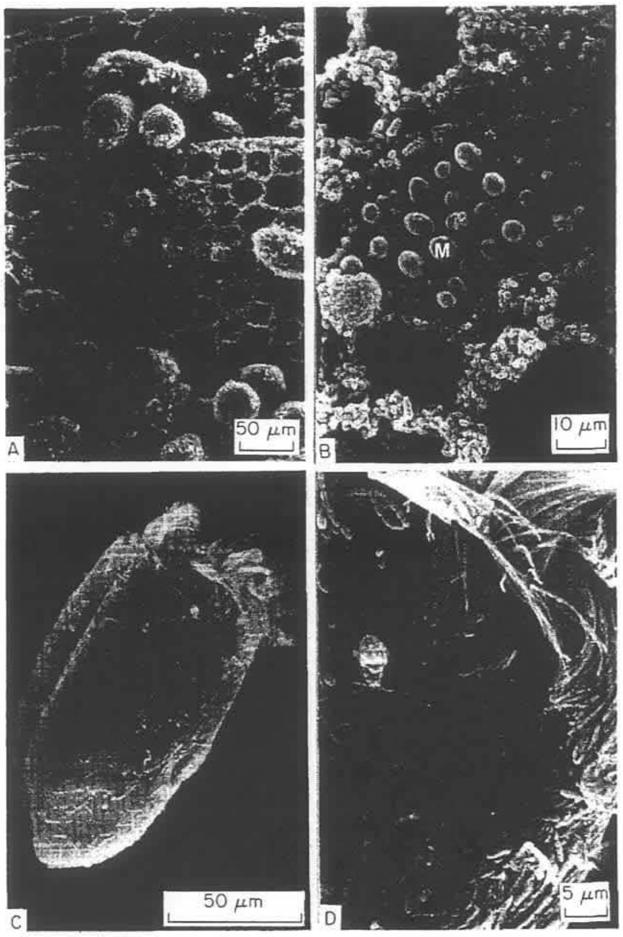

Fig. 3.3.4. Rumen micro-organisms. The 'invasion' of plant tissue by the protozoon *Isotricha intestinalis* (A) and bacteria, including what are probably cells of the large bacterium *Magnoovum eadii* (B), prior to digestion. The ophryoscolecid ciliates often carry bacteria attached to their external cuticle; this cell of *Eudiplodinium maggii* (C) has large numbers of rod-shaped bacteria, attached singly and in chains (D).

The animal mixes the rumen contents by regular contractions of the rumen wall. This mixing is of crucial importance to the efficiency of rumen fermentation since it serves to help inoculation with rumen micro-organisms, spreads the saliva throughout the rumen, enhances the absorption of fermentation products by replenishing the rumen liquor near the rumen epithelium, reduces the tendency of the plant fragments to float, and assists the mechanical breakdown of the fragments. Despite this, stratification of digesta does occur to some extent. The larger, less dense, little-digested fragments form an upper digesta layer, and the smaller, more dense, more digested fragments form successively lower layers. The least dense fragments can be returned to the mouth by eructation where they are fragmented by further chewing and grinding (rumination) and swallowed again. This action produces new fracture sites on the plant cell walls for microbial colonization. The smallest, most dense particles eventually leave the reticulorumen via the reticulo-omasal orifice with rumen liquor containing suspended micro-organisms. Turnover times for the liquid and solid phases of bovine rumen contents are 4–30 hours and 20–55 hours, respectively. The turnover time, which varies with the animal species, is influenced by the composition, rate of intake and particle size of the diet.

In the rumen, the micro-organisms produce essential nutrients for the host animal (see Fig. 3.3.5). The plant components are fermented principally to short-chain fatty acids, mostly acetate, propionate and *n*-butyrate, with the generation of carbon dioxide, methane and microbial cells. A number of other types of animal also have foregut fermentation; concentrations of volatile fatty acids are given in Table 3.3.4. In cattle, daily production has been calculated to be 3.7 kg of acetic acid, 1.1. kg of propionic acid and 0.6 kg of *n*-butyric acid, which yield 399 g of bacterial and 315 g of protozoon cells (dry wt) [162]. The fatty acids are absorbed directly through the rumen epithelium to serve as carbon and energy sources for the animal; the gases are lost by eructation. The microbial cells pass out of the rumen to the abomasum and remainder of the alimentary tract where they themselves are digested. Plant tissues passing from the rumen undergo secondary fermentation in the caecum and large intestine. In the caecum the secondary fermentation provides 8–30% of the total energy removed from the ingested feed. In contrast, ruminal fermentation provides 60–65% [52].

The extent of microbial digestion in the rumen is determined by the balance between the rate of fermentation and the rate of passage of the rumen contents. Rates of passage also affect the composition of the rumen microbial population since organisms with generation times greater than the turnover time wash out.

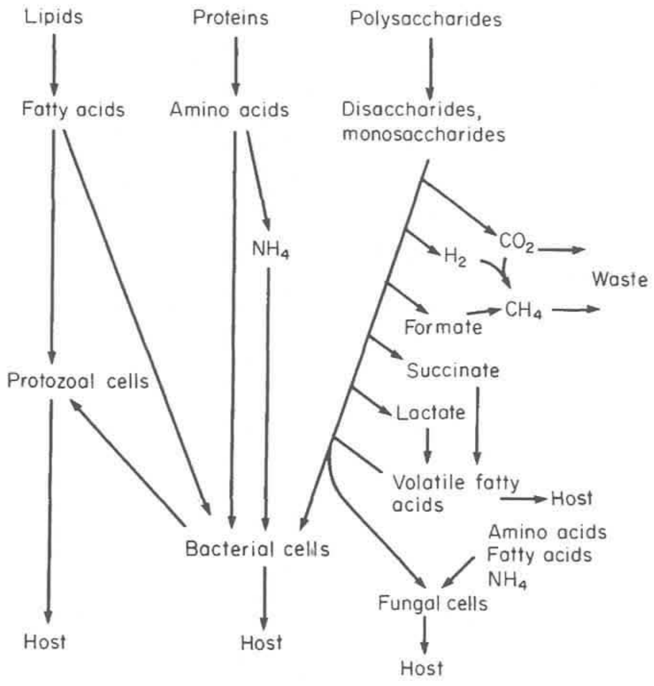

Fig. 3.3.5. Major metabolic activities occurring in the rumen.

THE MICROBIOLOGY OF THE RUMEN

The rumen microbial population is very complex, consisting of many different types of interacting eukaryotic and prokaryotic micro-organisms. The population is dominated by bacteria (10^{10}–10^{11} ml^{-1}), ciliate protozoa (10^4–10^6 ml^{-1}) and chytridiomycete fungi; flagellate protozoa, amoebae and bacteriophages also occur. In addition, there are many types of micro-organisms which have been observed in the light microscope and electron microscope that have yet to be identified. Reviews of the biochemistry and ecology of rumen micro-organisms are available in [62, 63, 66, 108, 121, 122].

Table 3.3.4. Volatile fatty acid concentrations and pH in forestomachs of some animals with pregastric fermentation chambers*

	Volatile fatty acids, mM				
	Total	Acetate	Propionate	*n*-butyrate	pH
Colobid monkeys	103–219	50–64	22–28	6–14	5.5–7.0
Cattle	56–224	45–70	9–30	7–30	5.5–7.0
Sheep	32–187	38–69	15–30	9–17	5.5–7.0
Sloth	35– 95	—	—	—	5.2–6.7
Hippopotamus	60–180	—	—	—	5.0–5.7
Macropod marsupials	101–137	71–85	11	17	4.6–8.0

* Modified from Bauchop [9] and Hungate [66].

The rumen micro-organisms live in three distinct but interacting habitats — the rumen liquor, the digesta fragments and the rumen wall. Many of the micro-organisms are obligate anaerobes found only in the rumen or gastro-intestinal tracts of mammals, but facultatively anaerobic bacteria are also present. Some of these bacteria, particularly *Streptococcus faecium*, may play a significant role in ureolysis on the rumen epithelium. Possibly the major role of facultative anaerobes in the rumen is to scavenge oxygen and initially to aid the establishment of the rumen flora; subsequently they help maintain a low redox potential in the system. Some of the most common rumen micro-organisms, their major substrates and their fermentation products are given in Table 3.3.5.

CELLULOLYSIS

The bulk of the diet of ruminants consists of cellulose (30–60%) and hemicellulose (30–50%), and it is on the hydrolysis of these polymers that most of the microbial growth is dependent, either directly or indirectly. As in all other ecological niches, cellulose decomposition or digestion is generally carried out by a 'cellulase complex' consisting of three classes of hydrolytic enzymes: endoglucanases (C_x-cellulases), cellobiohydrolases (C_1-cellulases) and cellobiases (β-glucosidases). The endoglucanases attack amorphous (partially hydrated) regions on the surface of crystalline cellulose and create 'nick' sites. This releases cellobiose, exposes additional sites for attack

Table 3.3.5. Physiological niches, substrates utilized and fermentation products of more abundant rumen micro-organisms*

Organism/niche	Substrates fermented	Major end products of fermentation
Cellulose degradation		
Bacteria		
Bacteroides succinogenes	Cellulose, cellobiose, glucose	Succinate, acetate, formate
Ruminococcus spp.	Cellulose, cellobiose, xylose	Succinate, lactate, acetate, formate
Butyrivibrio fibrisolvens	Cellulose, widely adapted	Butyrate, lactate, formate
Protozoa		
Epidinium ecaudatum	Cellulose, hemicellulose, starch	Acetate, butyrate
Ophryoscolex sp.	Cellulose, hemicellulose, starch	Acetate, butyrate
Fungi		
Neocallimastix frontalis	Cellulose, hemicellulose	Acetate, lactate, formate, ethanol
Pectin degradation		
Bacteria		
Lachnospira multiparus	Pectin, cellobiose, glucose	Formate, lactate, acetate
Succinivibrio dextrinosolvens	Pectin, maltose, xylose	Acetate, succinate, lactate
Starch degradation		
Bacteria		
Streptococcus bovis	Starch, soluble sugars	Lactate
Bacteroides amylophilus	Starch, maltose	Succinate, acetate, lactate
Protozoa		
Entodinium caudatum	Starch	Acetate, butyrate
Methane production		
Bacterium		
Methanobacterium ruminantium	$H_2 + CO_2$, formate	Methane
Miscellaneous		
Bacteria		
Selenomonas ruminantium	Soluble sugars	Acetate, propionate
Megasphera elsdenii	Lactate, some sugars	Range of volatile fatty acids
Bacteroides ruminicola	Range of sugars	Succinate, acetate, formate
Protozoa		
Isotricha intestinalis	Sugars	Lactate, butyrate, acetate

* Modified from Hungate [66] and Ogimoto & Imai [108].

by endoglucanases and generally disrupts the structure of the crystalline cellulose matrix. The cellobiose is hydrolysed to glucose by cellobiase. The endoglucanases and cellobiase (as well as other α-glucosidases) are widespread among animal groups and many claims of cellulolytic activity in guts are based on assays using carboxymethyl cellulose (CMC) or other forms of hydrated cellulose. However, efficient cellulose digestion must involve cellobiohydrolases to denature crystalline cellulose, though comminution of the plant material will serve to disrupt the cellulose matrix to some extent. Other enzymes, for example pectinase, may also be important in exposing cellulose fibrils to enzymic attack. All these classes of enzymes may occur in the same microbe, for a cellulosome complex, providing complete 'cellulase' activity, has been isolated from *Clostridium thermocellum* [12].

Relatively few of the rumen bacteria are truly cellulolytic. The most common are *Bacteroides succinogenes*, *Ruminococcus albus* and *R. flavefaciens*. Some strains of *Butyrivibrio fibrisolvens* are also cellulolytic, particularly in ruminants living at nutritional extremes, such as the high-arctic Svalbard reindeer [114] and starving zebu cattle fed poor forage [86]. In *Bacteroides succinogenes* and the cellulolytic *Ruminococcus* spp. attachment by glycocalyx to the cellulosic substratum is a prerequisite to cellulolysis by cell-wall bound bacterial enzymes. In *B. fibrisolvens* attachment of this type does not occur, and this species forms satellite microcolonies around plant particles outside the glycocalyx formed by the attached cellulolytic species [36]. It is likely that these species release cellodextrins and cellobiose produced during cellulolysis into the rumen liquor, where they may be fermented by a variety of other bacteria including *Selenomonas ruminantium*, non-cellulolytic *Bacteroides* spp. and *Streptococcus bovis* [130]. This fermentation probably supports continuing cellulolysis by the removal of compounds that inhibit the activity of cellulases by end-product inhibition.

The anaerobic chytridiomycetes are a recently recognized group of micro-organisms, so far found only in the alimentary tracts of herbivores. The life cycle of rumen species consists of an alternation of generations between the motile flagellated zoospore stage, free in the rumen liquor, and the vegetative, reproductive stage (see Fig. 3.3.6), which grows on the digesta fragments [10, 110, 111, 113]. All the species so far isolated from the rumen are cellulolytic, and their populations are higher in animals fed high-fibre diets than in those fed concentrates or at pasture. Growth of the fungi on plant fragments weakens and disrupts the tissues, which probably allows the rumen bacteria greater access to the interior of the fragment and thereby accelerates the digestion.

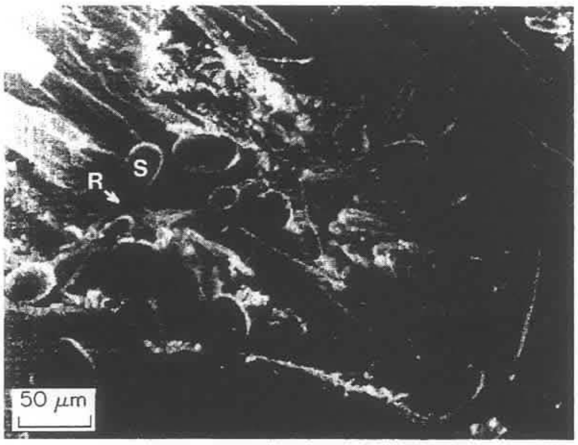

Fig. 3.3.6 The anaerobic rumen fungus *Neocallimastix patriciarum* growing on plant tissue *in vitro*. The rhizoids (R) and sporangia (S) are clearly visible.

Many species of ophryoscolecid ciliates ingest fibrous plant tissues. Since these ciliates always contain ingested bacteria, and bacteria may live within the cell and on the external cuticle [41, 68, 154] (see Figs. 3.3.4C, D), there has been some dispute as to how much of the cellulolytic activity associated with the ciliates is truly of protozoal origin. It is probable that the protozoa produce some cellulase, since in *Eudiplodinium maggii* (see Fig. 3.3.4C) at least 70% of the cellulase was found not to be of bacterial origin [42]. However, the most abundant protozoan genus, *Entodinium*, is not cellulolytic.

Bacteria are often attached to the external cuticle of ophryoscolecid ciliates (see Figs. 3.3.4C, D). Two bacterial types, methanogens [154] and *Ruminococcus albus* [68], have been shown by immunofluorescence to attach to ciliates, but other morphological types are common. The significance of this interaction has yet to be demonstrated, but the methanogens may stimulate the growth of the ciliate by removing hydrogen evolved during fermentation.

Hindgut fermentation systems

In the Equidae (horses and related species) microbial fermentation for their energy supply takes place in the large intestine (the horse has a 25–30 l caecum and a 55–70 l colon). The process is similar to that in the rumen and volatile fatty acids formed are absorbed through the caecum wall.

Two main features distinguish hindgut fermentation from rumen-type fermentation systems. First, in non-ruminants, the microbial substrates have already been exposed to the digestive enzymes of the host animal prior to fermentation, and, second, the microbial cells formed in the hindgut cannot be directly utilized by the animal and are excreted in the faeces. The result of the loss of the microbial cells is that herbivores relying on caecal or colonic fermentation generally have higher dietary requirements for preformed amino acids and vitamins than ruminants. Losses of nitrogen and vitamins are reduced by some mammals (e.g. rabbits) which practise coprophagy of special soft faecal pellets [64]. These pellets accumulate in the stomach where they inoculate freshly ingested food prior to passage to the caecum.

Numerous other mammals, birds, reptiles and possibly some fish have pouches in their large intestine or caecum which house large microbial populations [82, 125]. The significance of these populations in many species has yet to be determined. Some birds, however, use the volatile fatty acids generated by caecal microbes in response to urea which passes through the caecal wall [92].

Host–micro-organism interactions

The epithelium lining the rumen, caecum and large intestine in many animals is the site of regulation of the uptake of volatile fatty acids and the release of urea. A part of this process is due to the bacterial populations which are attached to the epithelium [36]. Some populations are very host specific; for example, filamentous adherent micro-organisms colonizing the ileum of mice will not colonize the ileum of rats and vice versa [148]. In the rumen some of the adherent bacteria are ureolytic facultative anaerobes which may regulate urea flux across the rumen epithelium [38]. Micro-organisms adherent to the wall of the bird and horse caeca are believed to have a similar function. In addition, the adherent facultatively anaerobic population aids in the maintenance of rumen anaerobiosis by utilizing blood oxygen after it diffuses through the epithelium. Another type of adherent cell digests the protein of dead epithelial cells. Although the adherent bacterial population is in tight contact with the epithelial cells of the rumen, antibody production to these bacteria occurs not in the rumen wall but in the caecum wall, and possibly the wall of the colon and small intestine [138]. The rumen micro-organisms *per se* do not appear to be pathogenic to the host, and they may play a role in protecting the host against pathogens by stimulating the production of cross-reacting antigens.

Adherent populations sometimes affect the structure and topography of the epithelium cells [133, 134] or modify chemical receptors on the surface, which controls the selection of species [56].

Intermicrobial relationships

The microbial attack and digestion of plant particles in the rumen is by a consortium of species. The cellulolytic species adhere to the particles in close contact with the cell wall polymers; satellite microcolonies of non-adherent, non-cellulolytic and cellulolytic species occur nearby [36]. Fermentation products, such as hydrogen, formate, succinate, ethanol and carbon dioxide which are produced by the adherent polysaccharide fermenters, may be utilized by the satellite species in addition to hexoses and cellodextrins which may be released by the cellulolytic species. When these ideas were tested in the laboratory, it was found that growth in co-culture could increase bacterial growth yield and change the pattern of fermentation products [161]. For example, succinate generated by *Bacteroides succinogenes* during growth on cellulose was used as an energy source by *Selenomonas ruminantium*, and hydrogen generated by *Ruminococcus flavefaciens* was used in methanogenesis by *Methanobacterium ruminantium* [79].

Antagonistic relationships occur, particularly involving the predation of bacteria by ciliate protozoa. The oligotrich protozoa of the genera *Entodinium*, *Polyplastron*, *Ophryoscolex* and *Epidinium* are the most active, and, in an average bovine rumen, may be responsible for the ingestion of 180 g of bacterial biomass per day [41]. Much of the bacterial nitrogen is released into the rumen fluid as free amino acids.

Interprotozoal predation also occurs as large species, such as *Polyplastron multivesiculatum*, ingest *Epidinium* spp. and *Entodinium bursa* ingests smaller *Entodinium* spp. An interesting result of the latter behaviour is the development of defensive caudal spines by the small entodinia in the presence of *E. bursa* [43].

3.3.4 INVERTEBRATE GUT SYSTEMS

There have been few studies on the role of micro-organisms in invertebrate guts in which all components have been functionally integrated at a level of understanding comparable to that of the rumen. Termites and cockroaches are exceptions and with certain of these species extensive research has been stimulated by applied problems of their pest status and control. The role of micro-organisms in the digestion of plant polysaccharides and the nitrogen balance of termites is therefore used as a framework for considering other invertebrate systems.

The invertebrate gut as a habitat

The food, feeding mechanisms, gut structure, gut physiological environment and digestive processes exhibit enormous differences both within and between classes, orders and even families of invertebrates. At the same time the gut microbiota range from symbionts with specific nutritional roles, through commensal gut bacteria, to ingested micro-organisms which survive gut transit to varying degrees. Ingested micro-organisms consist of free-living micro-organisms which may be selected as food or be taken in with other food materials. In both instances they encounter a sequence of events during gut transit which protects the indigenous micro-organisms from competitive displacement, determines the status of the animal as a carriage and dispersal agent for fungal and bacterial inocula and influences the susceptibility of the host to infection by pathogens.

Feeding

The first factor affecting the ingestion of microbial cells is the habitat occupied by the animal. Soil and litter animals, for instance, show a food preference related to the availablity of items in microhabitats occupied at any instant in time [116, 153]. Thus individual mites of the same species may have totally different gut contents even though they occupy soil or litter microhabitats separated by only a few millimetres [1].

The proportion of bacteria, fungi and other food materials ingested by invertebrates is also a function of size. Protozoa, for example, are able to selectively feed on bacterial cells or individual fungal spores or hyphae, while, at the other extreme, large litter-feeding invertebrates ingest fungi and bacteria in the proportions in which they occur at the feeding site. On the other hand, some fairly large invertebrates, such as certain beetle and fly larvae, are entirely mycophagous as they feed on or in large fungal fruiting bodies.

Structure and physiological environment of the gut

Although the gut of invertebrates differs markedly in structure and function between groups, three regions are evident on morphological and histological criteria: the foregut (stomodaeum), midgut (mesenteron) and hindgut (protodaeum). The relative proportions of these regions varies with the species, but the hindgut is often particularly large and elaborate in saprovores.

In arthropods the foregut and hindgut are ectodermal in origin and are lined with a cuticle which is shed on ecdysis. The gut lining in these regions offers potential attachment sites for micro-organisms and the hindgut walls are often heavily colonized by bacteria; spines or cuticular folds may prevent washout of symbionts (Fig. 3.3.7). Permeability of the foregut and hindgut walls is restricted by the cuticle, but invertebrates with basic nutritional dependence upon hindgut symbionts have patches or invaginations with thin epicuticle to facilitate the absorption of microbial fermentation products [17].

The midgut is endodermal in origin, is uncuticularized and is the main digestive and absorptive surface. In the majority of arthropods the midgut is lined with a delicate chitinous peritrophic membrane which protects the cells of the gut wall from abrasion by gut contents. The peritrophic membrane is continually being renewed and is thus an ephemeral surface for microbial attachment.

As in vertebrates, the chemical environment of the gut is affected by the nature of the ingested food material, the activity of the indigenous microbiota and substances secreted into the lumen. In most invertebrates the gut pH falls within the physiological optima (pH 6–9, see Table 3.3.6) for bacteria [17] with more acidic conditions prevailing in foreguts; midguts and hindguts are generally more alkaline. The survival of ingested bacteria may be affected by extreme conditions; for example, *Escherichia coli* is inactivated by the acid midgut (pH 3.0–3.5) of the blowfly larva *Lucilia* and bacteria present in this region are limited to a few species of spore-forming bacilli [58].

Little information is available on the redox conditions in invertebrate guts except for wood-eating insects. In nine species of termites both the foreguts and midguts were aerobic with an E_h in excess of +100 mV whereas the paunch and colon, where fermentation occurs, were anaerobic with E_h as low as −270 mV [152]. In the larva of the moth *Tineola* feeding on keratinous materials (wool, hair, feathers), the formation of cysteine and hydrogen sulphide decreases the redox potential in the gut to −200 mV [58] compared to +200 to +300 mV for many other insects [17].

In addition to enzymes and sloughed cells, the wide spectrum of organic and inorganic compounds secreted into invertebrate guts provide favourable conditions for

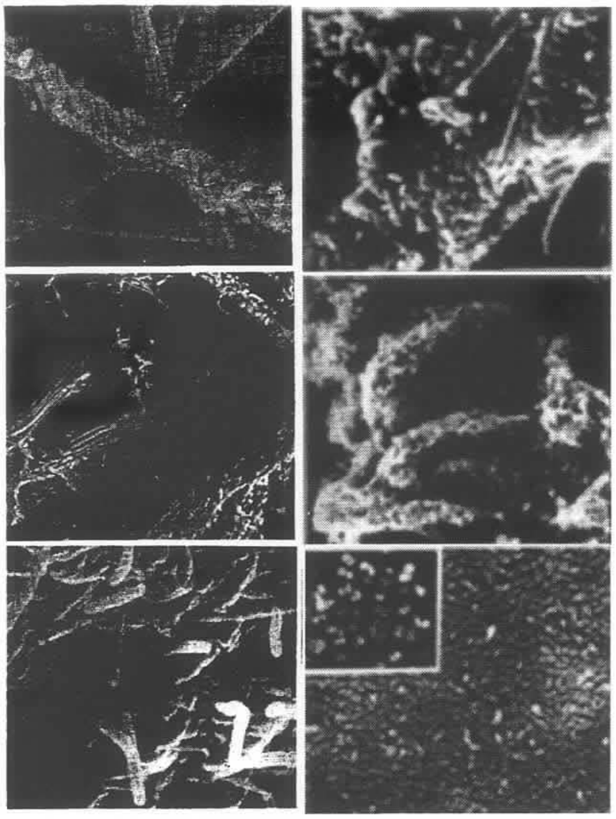

Fig. 3.3.7. Patterns of microbial attachment to cuticle. (A) On flat, relatively permeable surfaces a monolayer of rods or coccobacilli is most commonly encountered. *Schistocerca gregaria* (locust), colon. The inset shows coccoid forms which are occasionally encountered in the foregut. (B) End-on attachment is sometimes employed on less permeable cuticles, for example crustacean hindgut. It would be a suitable orientation for spore-formers or serve to remove parts of the cell from non-stirred layers adjacent to the surface. *Oniscus asellus* (woodlouse), hindgut. (C) Elongated spines may be furnished by the host as attachment surfaces for symbiont organisms, ensuring that the microbes come into intimate contact with food material in transit. Posterior colon of *Procubitermes aburiensis* (soil-feeding termite) showing filamentous prokaryotic organisms adhering to spines. (D) An alternative form of spine, branched distally, serving as an attachment site for long rods and coccoid couples. Ileum of *Acheta domestica* (house cricket). (E) Filaments may also attach to flat cuticular surfaces although a clearly differentiated hold-fast is not apparent. Posterior hindgut of *Cylindroiulus* sp. (millipede). (F) Epibionts may be found in some locations, suggesting secondary symbiotic relationships amongst the constituents of a gut flora. Hindgut of *Tachypodiulus* sp. (millipede). After Bignell [17].

bacterial growth. The main ionic fluxes are K^+, Na^+ and Cl^- associated with fluid inputs from Malpighian tubes and other excretory organs. Nitrogenous compounds excreted into the gut include uric acid, allantoin, urea, ammonia, xanthine and proteinaceous materials [40]. Microbial exploitation of these compounds may facilitate nitrogen conservation by the animal as well as make up any dietary deficiencies of essential amino acids [23]. Finally, mucus secreted into the gut of molluscs and earthworms, and from the Malpighian tubes of arthropods, forms a readily metabolizable carbon source for gut micro-organisms. The addition of this readily metabolized material to the gut contents of tropical earthworms may prime the depolymerization of recalcitrant soil organic matter fractions by gut bacteria [7].

Digestive processes

In general terms the enzyme complements of invertebrate guts reflect the diet of the animal; predators show high protease and lipase activity while herbivores and saprovores show higher levels of carbohydrase activity. The assimilation efficiency of animals feeding on plant materials is generally inversely related to the lignin and cellulose content of the food. However, many fungi and some bacteria are very effective in utilizing cellulose and, to a lesser extent, lignin, as energy sources [165]. Some insect groups, notably termites [23], cockroaches [16] and the larvae of dung- and wood-feeding beetles [13, 14] have symbiotic associations with micro-organisms which help digest these structural compounds.

The lower termites are predominantly wood-eaters and the assimilation efficiencies for cellulose and hemicelluloses (54–93%) are high in comparison with most other saprovores. There is some debate over the extent to which lower termites produce cellulases in addition to the gut protozoa, but quantitatively there is little doubt about the role of the symbionts. The hindgut paunch is the main site of polysaccharide digestion by the flagellate protozoa, which can constitute up to a third of the termite biomass. Wood fragments engulfed by the protozoa are fermented anaerobically to form acetic acid, hydrogen and carbon dioxide. About 80% of the acetate is derived from cellulose and 20% from hemicellulose [105]. While the protozoa do possess some cellulolytic activity not derived from endosymbiotic bacteria, they require bacteria as a source of nutrients or growth factors [164]; the hindgut bacteria therefore appear to be essential for the maintenance of the protozoa. The products of bacterial

Table 3.3.6. pH of the alimentary canal in representative arthropods [17]

Species	Foregut	Midgut	Hindgut
Insecta			
Periplaneta americana	4.8–6.8	6.1–6.6	6.3–6.8
Melanoplus bivattatus	5.5	6.7	6.4
Zootermopsis nevadensis	6.8	7.1	7.1
(Alkaline gut) (highest pH recorded)			
Culex pipiens and other mosquito larvae	—	10.5	7.0
Oryctes nasicornis	8.5	10.4	6.7
Cubitermes severus	6.7	7.5	10.4
Various Lepidoptera	—	10.3	8.9
Tipula abdominalis (larva)	—	11.6	—
(Acid gut) (lowest pH recorded)			
Lucilia cuprina	7.3	3.3	7.8
Various Diptera	5.2	2.8	7.3
Crustacea			
Calanus finmarchicus	—	6.0–8.0	—
Daphnia magna	—	6.0–6.2	—
Ligia oceanica	—	6.4	—
Astacus fluviatilis	4.2–6.6	—	—
Cancer pagurus	5.8–6.0	—	—
Acari			
Phthiracarus sp.	—	5.4–6.6	—

fermentation (lactate, butyrate and acetate) are absorbed by the termites (see Fig. 3.3.8) for respiration and biosynthesis. Assimilative carbon dioxide reduction is also proposed as a sink for carbon dioxide produced by fermentation [23] together with the production of methane by methanogenic bacteria as in the rumen. Rates of methane emission from termites under laboratory conditions may, however, exceed those of ruminants on a weight-specific basis [23], and it has been estimated that termites could be a major biogenic source of atmospheric methane [166]; this, however, is disputed [45].

Far less is known about the role of gut micro-organisms in the digestive processes of higher termites. It was shown [104] that 19% of the cellulase activity in *Nasutitermes exitiosus* was located in the foregut, 58% in the midgut, 14% in the mixed segment and 8% in the hindgut. Removal of the gut flora with tetracycline did not affect the location or activity of the enzymes, suggesting that the termite did not depend upon the gut flora for cellulose digestion. This type of experiment is, however, complicated by the phenomenon of acquired microbial enzymes, as demonstrated in woodlice [59] and other insect–fungus associations [89].

In the fungus-growing Macrotermitinae the basidiomycete *Termitomyces* sp. produces small spherical conidiophores on the surface of the fungus comb and these are selectively consumed by the termites together with comb material. In the midgut of *Macrotermes natalensis* all three classes of cellulases are present, while the anterior hindgut, where there are high numbers of bacteria, contains mainly endoglucanase and cellobiase. Extracts of the salivary glands and midgut tissues actively degraded carboxymethylcellulose but not microcrystalline cellulose, showing that the termites themselves could produce the endoglucanases but not cellobiohydrolases. In contrast, there was high activity of all types of cellulase in the nodules on the fungus comb. The midgut of the termite, an active site of cellulose digestion, contains the enzyme complex acquired from the fungus as well as low levels of animal cellulases [89].

NITROGEN

A general feature of mycophagy and bacteriophagy is that animals are able to acquire a high-quality food derived from resources deficient in nitrogen. This is particu-

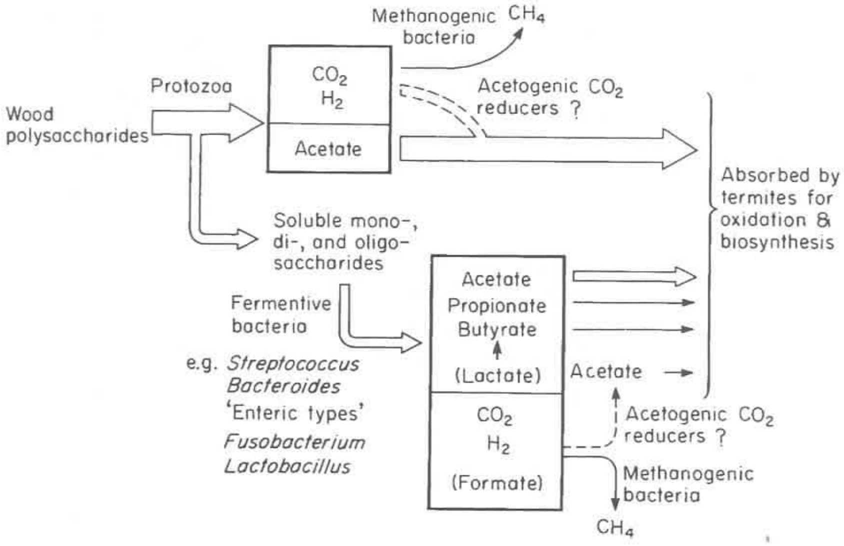

Fig. 3.3.8. Proposed working model for wood polysaccharide degradation in the hindgut of lower termites. Thickness of arrows represents approximate relative quantitative contribution of the respective reactions. Major products of the hindgut fermentation are indicated in boldface letters. After Breznak [23].

larly important in animals exploiting wood as food [144] and in the fungus-growing termites [44]. The woody materials collected by the termites contain less than 1% nitrogen and the nitrogen concentration in the fungal comb is 1.4%; the conidiophores, however, contain up to 38% protein and provide all the amino acids required for termite growth and reproduction [126]. Excretory nitrogen from the termites deposited on the combs as uric acid is also recycled by the fungus.

Uric acid is the major nitrogenous excretory product of terrestrial invertebrates because it can be voided in a crystalline form and thus minimizes water loss. In the cockroach *Periplaneta americana* uric acid accumulated in fat bodies when the animals were fed a high nitrogen diet and this store was mobilized when nitrogen was scarce [96]. The mechanism of nitrogen mobilization was not determined but, since insects lack uricase, a microbial intermediary was implicated. This has been definitively demonstrated [119] for the lower termite *Reticulitermes flavipes* where uricolysis occurs as an anaerobic process carried out in the hindgut by *Streptococcus* sp., *Bacteroides termitidis* and *Citrobacter* sp., the uric acid being fermented to ammonia, carbon dioxide and acetate. Uric acid was also transported in Malpighian tubules [119] from its site of synthesis and storage in fat bodies to the gut; microbial uricolysis *in situ* also released nitrogen that was re-used by the termites for biosynthesis. The same processes may occur in the millipede *Glomeris marginata* [4] and the endosymbionts of anobiid beetles may also utilize uric acid [5].

Dinitrogen fixation in invertebrate guts has been demonstrated in a wide variety of animal groups from many habitats, particularly in animals feeding on nitrogen deficient material, such as wood. Most determinations have been carried out on termites, and results show enormous variability in rates of dinitrogen fixation. These rates have been expressed in terms of the 'time to double nitrogen' (TDN) in the body of the termite [23] and it has been concluded that TDN values range from 6 months to more than 2000 years. Feed selection by the animals is probably more important in providing dietary nitrogen. The extreme variability of the results may also be due to disturbance factors or variable demands during growth, for example, in marine shipworms [29].

The gut microflora

Much information is available on the qualitative and quantitative characteristics of bacteria in the guts of aquatic animals [e.g. 76, 135]. Most of these studies show that aquatic invertebrates are similar to terrestrial invertebrates and contain a range of unicellular and filamentous bacterial morphotypes, occurring in similar attachment sites in the gut. Few examples of fungal commensals or dispersal of fungal inocula have been recorded in freshwater or marine environments, though algal symbiosis is common [141]. The following account is therefore based on terrestrial examples which illustrate the full spectrum of these invertebrate–microbial relationships.

Termites are well known for their nutritional dependence upon symbiotic protozoa but the majority of termites belong to the family Termitidae or 'higher termites', which harbour bacteria in their guts. Even in the 'lower termites' with protozoan symbionts, key metabolic processes are mediated by bacteria [22]. The paunch (anterior proctodaeum), which contains the protozoa, also holds a heterogeneous assemblage of bacterial cells. Many of these have hold-fast elements to secure them to the gut wall or an elongated growth form which may prevent washout.

The soil-feeding termites have a gut microbiota of unparalleled complexity with many novel features. For instance, in *Cubitermes severus* and *Procubitermes aburiensis* the midgut is heavily colonized by a diverse assemblage of bacteria including spirochaetes inserted between the microvilli of the mesenteric tissues. The interface between the midgut and the hindgut forms a 'mixed segment' which was found to have a carpet of spirochaetes of such uniform morphology as to suggest a pure culture [18]. These bacteria may contribute to the low redox conditions (−104 mV) in the large hindgut by removing oxygen from the digesta [10]. Spirochaetes, free-living as well as attached, appear to be of general occurrence in termite guts [22] and form spectacular 'motility assemblages' in the termite *Mastotermes darwiniensis* where co-ordinated undulations of spirochaetes attached to the protozoon *Mixotricha paradoxa* provide propulsion for the cell [39].

The hindgut of *Procubitermes* and *Cubitermes* is densely colonized by bacteria but in addition the posterior hindgut has dense wefts of actinomycetes, containing spirochaetes and other bacteria, attached to paddle-shaped cuticular spines (see Fig. 3.3.7). These associations are not unique, for scanning electron microscopy has revealed filamentous bacteria, probably actinomycetes, to be a dominant feature in the guts of many terrestrial invertebrates (see Fig. 3.3.7).

The protection of the gut microbiota, and indeed the organism itself, from invasion by ingested micro-organisms may be maintained by physical or biological mechanisms. The earlier example of *Lucilia* larvae, which maintain a highly acid foregut, is analogous to the acid stomach conditions in many mammals which lyse ingested bacteria. In the larvae of the wax moth *Galleria mellonella*, *Streptococcus faecalis* is the only bacterium present in high populations in the gut. *Streptococcus faecalis* suppresses the growth of bacteria ingested with food by the production of a bacteriocin as well as a bacteriolytic enzyme [73]. Such tight regulation of the gut microbiota may not be a general phenomenon in invertebrates, but may occur in animals with specialized diets where the bacteria have specific nutritional roles.

The faeces of soil animals such as millipedes, woodlice, fly larvae and earthworms may contain more than 500 times more viable bacteria per gram than the food materials on which the animals are feeding [69]. However, there are few invertebrates where the gut microbiota has been differentiated into free-living and indigenous bacteria.

Several studies of earthworm gut bacteria suggest that essentially the same groups are present as in the soil in which the animals are living [115, 131]. On the other hand, in earthworms feeding on rotten wood, it was found that 73% of 473 bacterial strains isolated from the gut contents belonged to the genus *Vibrio* (including pathogenic strains such as *V. cholerae*, *V. parhaemolyticus*, and *V. alginolyticus*) [87].

A similar increase in counts of viable bacteria during gut transit in arthropods has been recorded for many invertebrate groups [78] including woodlice [69, 123], fly larvae [146] and millipedes [2, 6, 145]. The calculated generation times for bacteria in the guts of the litter-feeding millipede *Glomeris marginata* are about 4 hours [69]. Actual generation times may be less than this because there are regions of the gut where a significant proportion of the ingested bacteria are lysed: approximately 53%, 53% and 29% of *Pseudomonas syringiae*, *Erwinia herbicola* and *Escherichia coli*, respectively, were assimilated by the millipede when fed to the animals on leaf discs [3]. Analysis of isolates from the food, guts and faeces of the millipede revealed that, although there was some overlap, the bacterial floras of the food litter and guts were distinct [69]. Similar results were obtained for the woodlouse, refuting other work [20] suggesting that the guts of these animals are sterile (see also Fig. 3.3.7). The majority of isolates from the food litters of *Oniscus* and *Glomeris* were *Pseudomonas* spp. The most predominant member of the gut population of *Oniscus* was *Klebsiella pneumoniae* and this organism was also a common isolate from the faeces of both animals [69]. The ability to degrade uric acid or urea is a characteristic of bacteria isolated from insect guts and was also found in *Glomeris* and *Oniscus* gut isolates. While this characteristic may be selected by the excretory products of *Glomeris* [4] it is not selected by those of *Oniscus*.

In studies on the gut flora of another millipede, *K. pneumoniae* was identified [6], together with members of the genera *Sarcina*, *Bacillus* and *Corynebacterium*; *K. pneumoniae* was also associated with the *Vibrio* complex of *Eiseria lucens* [87]. If the occurrence of *K. pneumoniae* is widespread in the guts of saprotrophic animals, this could resolve the anomalous occurrence of this coliform in rotten wood, sawdust and living trees [69]. It is not thought, however, that invertebrates are often vectors of pathogens of medical importance. There are few diseases where transmission of free-living bacterial pathogens to man by invertebrates is a significant facet of the epidemiology of the disease, reviewed in [25]. Notable exceptions are the carriage of the enterobacteria *Shigella* (bacterial dysentery) and *Salmonella typhi* (typhoid) by flies. The bacteria pass rapidly through the guts but persist in the lumen for up to 7 days. Transmission occurs by 'vomit drops' or frequent defaecation.

The most notorious insect-transmitted diseases are typhus and bubonic plague, which are parasites of specific vectors. Typhus is caused by *Rickettsia prowazeki*, which multiplies in the gut of the louse after being ingested with a blood meal from an infected host. The bacteria remain virulent in dry louse faeces for at least three months. Infection occurs when this material is inhaled, rubbed into mucous membranes or scratched into the skin. In bubonic plague, the bacterium *Yersinia pestis* proliferates in the gut of the flea and more or less blocks it. The flea is unable to take in the next blood meal and as blood is sucked up, it spurts back into the host, carrying with it detached clumps of bacteria. The infection is generally fatal to both host and vector.

The occurrence of fungal spores in the guts of soil animals has been comprehensively reviewed [153]. A very diverse group of invertebrates and fungi are involved, though there are few cases where dependence on invertebrate vectors for dispersal has been demonstrated. Spores of many wood-decomposing fungi were present in the guts of woodlice [147] but spore viability of *Merulius lacrymans* was reduced after egestion. In contrast it has been found that it was essential for one spore type of a basidiomycete to pass through the gut of a fly larva before it would germinate [103].

The gasteromycete fly *Phallus impudicus* is a good example of a vector for spore transmission. These flies are attracted to the odour of the sticky spore slime on the fungal cap. The spores pass through the gut of the flies unharmed. Several types of fungi have evolved dispersal mechanisms which involve attracting flies or other insects to feed on sugary secretions containing spores. In *Claviceps purpurea* (which produces ergots on rye and other members of the plant family Graminaceae), primary infection of the host flowers is by wind dispersal of ascospores but the ovary of the plant is converted into a mass of fungal conidiophores which secrete a noxious-smelling, sugary secretion containing conidia which are dispersed to healthy plants through insect guts [70].

Most instances of transmission of fungal spores in invertebrate guts involve passive mechanisms, but the fungus-growing termites carry inocula of *Termitomyces* between one colony and the next [74]. Reproductive stages of *Macrotermes* and five species of *Microtermes* carry *Termitomyces* conidia in their guts during the nuptial flight to start a fungus comb in the new colony. The winged stages of *Macrotermes sublyalinus*, *Ancistrotermes* spp. and *Odontotermes* spp. do not do this, however, and the combs appear to originate from basidiospores collected by the workers.

The entomopathogenic fungi provide interesting insights into the gut as an environment for micro-organisms [38, 159]. The buccal cavity, foregut and hindgut are the main sites of fungal penetration. Gut conditions, however, are generally unfavourable for spore germination. Conidia of *Metarhizium anisopliae* fail to germinate in the gut of the beetles *Oryctes rhinoceros* and *Hylobus pales* although they pass through the gut in a viable state. Conidia of *Beauvaria bassiana* were inhibited from germinating *in vitro* by gut contents of several insect species, a phenomenon attributed to high gut pH or nutritional unsuitability [35]. These conditions did not account for the failure of *M. anisophliae* conidia to germinate in the gut of locusts, where a heat-labile toxin, possibly produced by the gut bacterial flora, was implicated.

The Trichomycetes, a diverse but ecologically defined group of lower fungi, are obligate commensals of a very wide range of invertebrates [93]. The majority of species are found in the gut lumen and have fairly specific attachment sites in the foregut, in the hindgut and on the peritrophic membrane. All the species are characterized by a specialized hold-fast for thallus attachment and a life cycle adapted to reinfecting the host after ecdysis. Attachment of spores is rapid and the trichospores of *Smittium culisetae* become attached to the hindgut cuticle of mosquito larvae within 30 minutes of ingestion. There is little evidence of harm or benefit to the host from the association.

3.3.5 ORGAN-BOUND ASSOCIATIONS

Endosymbiosis, in which cells of the host contain micro-organisms, is widespread among insect groups with specialized diets such as blood, plant sap and wood. Nearly all anobiid and cerambycid beetles have yeast-like endosymbionts contained in gut caecae [75] and endosymbiotic bacteria, particularly rickettsiae, are found in at least 10% of all insects [158]. The endosymbionts of Homoptera show a particularly wide range of morphotypes [65] from typical rods to the bizarre 'c' and 't' symbionts of leafhoppers. Other notable bacterial associations occur in annelids and pogonophora, and micro-algae are found in many coelenterates and protozoa.

Endosymbiotic bacteria are found in the cells of the gut wall in some cerambycid larvae where they are constantly voided into the gut lumen. Generally, though, they are contained in specialized cells termed bacteriocytes. These may be aggregated (as in the midgut wall of tsetse flies, lice and ants of the genus *Camponotus*); located in modified Malpighian tubules (as in some plant-feeding weevils and crysomelid beetles); or scattered through fat bodies (cockroaches, most homopteran bugs, bloodsucking bugs and many wood-feeding beetles). The relationship between the endosymbionts and the host is highly specific and often monospecific. Consequently transfer between generations often occurs within the ovary. In the bed-bug *Cimex* (Heteroptera) the bacteria invade the nurse cells and hence are transferred to the eggs while in *Rhizopertha* (Coleoptera) the bacteria pass with sperm from the male to the female and enter the micropyle of the egg during fertilization [24]. In other species infection is via the gut. The eggs of anobiid beetles are smeared with secretions containing yeast from the glands around the ovipositor and the hatching larvae are infected when they eat part of the eggshell. The tsetse fly (*Glossina*) larva undergoes full development within the body of the female and the bacteria are transferred from 'milk glands' to the larval gut. Unlike ectosymbiotic associations, endosymbionts are not lost by ecdysis.

Studies on the functional significance of endosymbionts to the host have been carried out by eliminating the symbionts or by the maintenance *in vitro* of isolated symbionts and mycetocytes [34, 65]. It has been demonstrated that the symbionts can correct nutritional deficiencies in insect diets by producing various amino acids, sterols and B vitamins. For example, when larvae of the anobiid beetle *Stegobium* were devoid of symbionts, they were only able to develop with the addition to the diet of thiamin, riboflavin, pyrimidine, biotin, nicotinic acid, pantothenic acid, folic acid, choline and cholesterol [54, 55]. Symbionts of *Lasioderma*, however, could supply all the ten essential amino acids, but only lysine and phenylalanine, limiting in the diet, were in sufficient quantities for optimum growth [75].

Another role for endosymbionts is found in the leech *Hirundo medicinalis*, which depends upon the bacterium *Pseudomonas hirudinis* to lyse ingested erythrocytes in the gut caeca [124].

Despite the extensive surveys which have been carried out on bacterial endosymbionts, new and unusual functional relationships are still being reported. One of the most spectacular recent discoveries was the finding of chemoautotrophic bacteria in the Pogonophora. The Pogonophora are worm-like animals (deuterostomes) which do not possess a digestive tract (see Fig. 3.3.9). They are mainly found in the deep seas, consequently they were not discovered until the beginning of the 20th century.

In the late 1970s a remarkable discovery was made of 'giant', plumed pogonophora, subsequently named *Riftia pachyptila*, together with large clams and other fauna, clustering around hydrothermal vents at sites 2.5 km deep in the Galapagos Rift and the East Pacific Rise [72]. Normally benthic populations of animals are sparse at this depth because of the remoteness of photosynthetically productive surface waters. In this community, it has been shown that the primary nutrient and energy source is the chemoautotrophic production of bacterial biomass utilizing hydrogen sulphide emitted from volcanic vents [71]. *Riftia*, which are up to 1.5 m in length and 38 mm in diameter, are much larger than pogonophora from the north-western Pacific (10–35 mm) but the basic body structure is similar. Chemoautotrophic symbionts are found in bacteriocytes which line a fluid-filled cylinder at the core of the body [31, 32]. Transfer of metabolites is believed to be by bacterial excretion and uptake by host membranes, or possibly by lysis of bacteria. Evidence for the chemoautotrophic nature of the bacteria comes from electron micrographic studies which show the abundance of the bacteriocytes and the endosymbiotic habit. Detailed studies of European *Schlerolinum* and *Siboglinum*, which live in rotten wood and organic sediments with high sulphide content, have revealed similar bacterial structures (see Fig 3.3.10) as well as carbon dioxide-fixing ribulosebisphosphatecarboxylase, which also points

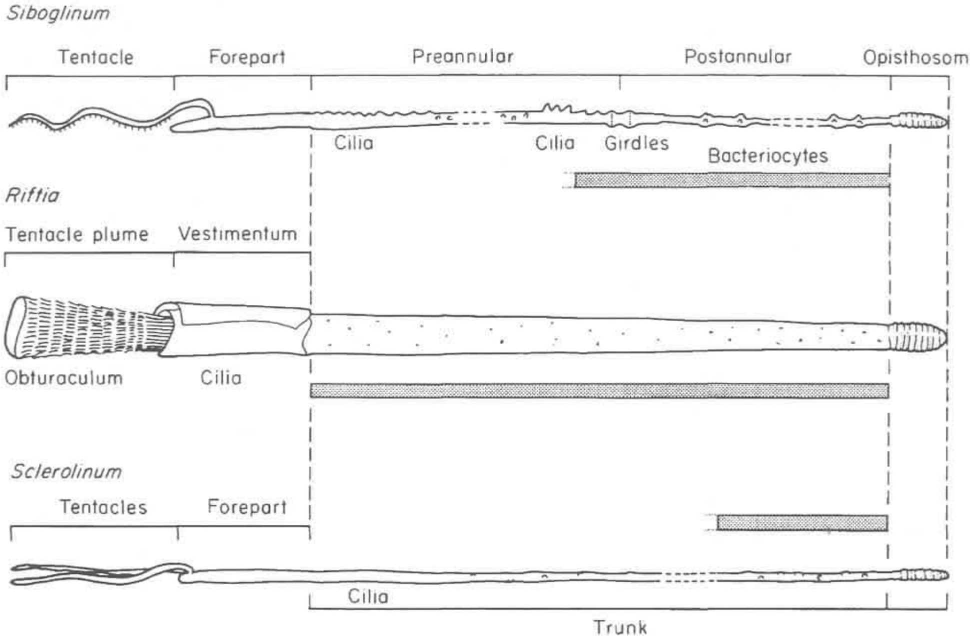

Fig. 3.3.9. Diagrams comparing body divisions in Pogonophora — much shortened. *Siboglinum*, pattern found in most Perviata; *Riftia*, pattern found in Obturata (Vestimentifera); *Sclerolinum*, pattern found in one family of Perviata (Sclerolinidae). From Southward [142].

to chemoautotrophic processes [142]. On the other hand the gutless clam, *Solemya reidi*, from sulphide-rich habitats, has gills containing symbiotic, chemolithotrophic bacteria, which were assumed to oxidize sulphide to provide the major energy source for the symbiosis. However, it was later found that the initial steps in sulphide oxidation occur in specific organelles in the gill cytoplasm and not in the bacteria [120]. This study illustrates the dangers of assuming functional roles of animal–microbial associations.

Another recent discovery is the occurrence of symbiotic bacteria in the gland of Deshayes in several species of shipworm. Shipworms are wood-boring marine bivalve molluscs in which the valves are reduced in size and used for boring tunnels in submerged wood. The body of the mollusc completely fills the tunnel and the animal feeds by ingesting wood fragments produced by the rasping action of the valves. The gland of Deshayes contains aerobic, nitrogen-fixing cellulolytic bacteria; these are believed to secrete cellulase into the gut of the mollusc to enable it to digest cellulose in the wood fragments. This gland is the only known example of an association between a cellulolytic bacterium and an organ that is not part of the digestive tract [155].

ALGAL SYMBIOSIS

A variety of aquatic invertebrates posses symbiotic algae. Coelenterates provide many examples such as the green hydra (*Hydra viridissima*) and reef-forming corals. Algal symbionts are also found in protozoa, platyhelminths, molluscs and freshwater sponges; altogether, about 150 invertebrate groups are known to be involved [141]. The algae, which are usually coccoid forms, are from three classes: Cyanophyceae, Chlorophyceae and Dinophyceae. Various types of associations between algae and invertebrates are summarized in Table 3.3.7.

The nutritional role of zoochlorellae in *Hydra viridissima* has been extensively investigated. The algae synthesize maltose, which is available to the *Hydra* for metabolism and growth [97].

The protozoon *Paramecium bursaria* typically has several hundred zoochlorellae per cell and again there is

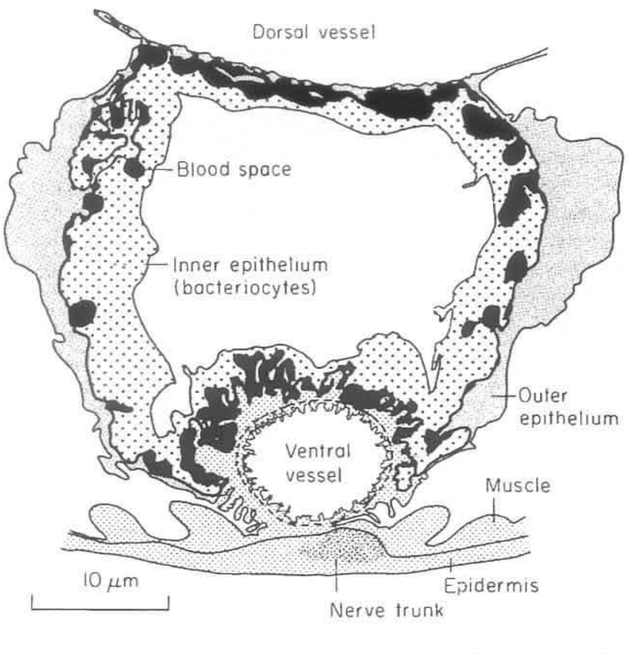

Fig. 3.3.10. Part of a transverse section of the postannular region of *Siboglinum fiordicum* showing the bacterial cylinder between the dorsal and ventral longitudinal blood vessels. Blood in the blood space shown black. From Southward [142].

algal strain recognition and carbon transfer as maltose. The photosynthetic activity of the algae facilitates the colonization of sediments with low oxygen tensions.

The zoochlorellae of corals are also important as oxygen suppliers since reef corals only do well in shallow waters where photosynthesis exceeds respiration [51]. It has often been suggested that the symbiotic algae assist in the carbon metabolism and calcification of corals. Tropical sea water is generally saturated with calcium bicarbonate and, as bicarbonate ions are removed from the water by algal photosynthesis, calcium carbonate is precipitated.

3.3.6 APPLICATIONS

Knowledge gained from understanding the microbial processes operating in the alimentary tracts of animals has far-reaching applications in the fields of nutrition, health and biotechnology. Fermentative digestion is only advantageous to an animal for utilizing substances that it is unable to digest using its own enzymes or which otherwise have poor nutritive value. Fermentation of protein, soluble carbohydrates and starch from the food, and hexoses and other products generated during plant cell wall digestion, inevitably leads to a loss of carbon, nitrogen and energy. The efficiency of animal production may be optimized by properly balancing the fermentation against the animal's own digestive capacity and requirements.

Ruminal fermentation of compounds that the animal itself can utilize in its lower alimentary tract can be controlled by protecting these components from the action of rumen micro-organisms and by controlling the rumen microbial species present or their activities. For example, protection of proteins from rumen fermentation has been achieved by treatment with heat, tannins and aldehydes [53]. Heat treatment, however, results in decreased digestibility of other dietary components, but treatment with uncondensed tannins causes cross-linking between proteins and other macromolecules which reduces microbial hydrolysis in the rumen.

Treatment of forages with formaldehyde protects protein from ruminal digestion without rendering it indigestible in the small intestine. Many chemical reactions occur when protein is treated with formaldehyde, but the major reactions — the formation of methylol groups on the terminal amino acids and the amino group of lysine, followed by condensations — are reversed under the acid conditions in the small intestine; this exposes the protein to enzymic digestion.

The amino acids methionine, cysteine, lysine and threonine are essential to the host animal, but are all subject to deamination in the rumen. Thus they may be growth-limiting when the animal is fed a poor-quality diet. Encapsulation of these animo acids in styrene copolymer matrices [53] reduces ruminal losses and allows significant quantities of these amino acids to be carried to the small intestine where some are absorbed. An alternative encapsulation method, coating nutrients with a layer of formaldehyde-treated protein, has proved successful for protecting unsaturated lipids from ruminal hydrogenation [137]. This method may be used to transport a variety of compounds (for example vitamins, hormones and pharmacological agents) to the small intestine.

Chemicals which inhibit methanogenesis have also been well studied and it was found that the ionophores monensin and lasalocid [33] and amicloral are partially successful. Monensin is currently used as a feed additive for cattle. The precise mode of action of monensin on methanogenesis is not known, but it is likely that it affects the generation of H_2 by non-methanogenic organisms, rather than the methanogens themselves. Monensin

Table 3.3.7. Summary of the different types of associations between algae and invertebrates found in sea water and fresh water [140]

	Sea water			Fresh water		
	Alga	Animal	Comments	Alga	Animal	Comments
Zoochlorellae	*Platymonas convolutae* (Pyramimonadales) ? Chlorophyceae	*Convoluta roscoffensis* (platyhelminth) *Anthopleura* sp. (sea anemone)	Zoochlorellae are uncommon in marine associations	*Chlorella* sp. (with some reports of *Scenedesmus*)	Protozoans Sponges *Hydra* Platyhelminths	The commonest type of freshwater association
Zooxanthellae	Dinoflagellates	Sponges Coelenterates Molluscs	Dinoflagellates are very common, especially in tropical oceans			None reported
	Cryptomonads	*Cyclotrichium* (ciliate)				
	Diatom	*Convoluta convoluta*				
Cyanellae	Unidentified blue-green algae	Echiuroid worms Sponges	Uncommon	*Synechococcus*	Protozoans	Uncommon
Chloroplasts	Siphonaceous seaweeds (*Codium*, *Caulerpa*, *Bryopsis*)	Molluscs (*Elysia*, *Tridachia*, *Tridachiella*, *Placobranchus*)	Previously described as zoochlorellae			None reported
	Cladophora	Molluscs (*Limapontia*, *Acteonia*)				
	? Red algae	Mollusc (*Hermaea*)				
	?	Rotifers				

treatment causes an increase in propionate and a decrease in acetate and *n*-butyrate concentrations in the rumen. This is advantageous for growing animals, which use the propionate for glucogenesis, but not for lactating animals, which rely on high ruminal acetate production for maximum lipogenesis in the mammary gland. It is also true that complete inhibition of methanogenesis is undesirable because many rumen organisms gain energy from the metabolic pathways involved in H_2 production; if there were no methanogens, the high ruminal H_2 concentrations would inhibit these pathways.

In the large intestine of man, microbial metabolism of ammonia and other nitrogenous compounds may be a cause of bowel cancer. To decrease the risk it has been recommended that diets contain a high level of fibre. The rationale is that a high fibre intake causes greater microbial activity and thus greater microbial nitrogen requirements in the large intestine. This results in low residual levels of free nitrogenous compounds in the bowel and also in relatively high levels of production of *n*-butyrate, an anti-tumour substance in animals.

In biotechnology, anaerobic fermentations have the major advantage that, unlike aerobic processes, they do not need expensive aeration and agitation. Understanding the reactions of gut anaerobes, both in pure culture and in stable co-cultures, has indicated a potential for the development of systems yielding products such as hydrogen, methane and ethanol from high-fibre agricultural wastes. For example, the rumen fungus *Neocallimastix frontalis* ferments straw and other fibrous plant tissues to a mixture of acetate, lactate, formate and ethanol. In co-culture with methanogenic bacteria, the major products are acetate and methane [94]. Thus, ethanol and methane can be produced from straw in a single culture. Similarly, *Methanobacterium ruminantium* may be co-cultured with the cellulolytic *Ruminococcus flavefaciens*, yielding methane, acetate and succinate from cellulose [79]. While these systems have not been used commercially, it is likely that, with improved strains of micro-organisms, large-scale plants could be operated digesting a variety of fibrous agricultural wastes. Similarly, genes isolated from gut anaerobes which degrade plant cell walls and cloned into organisms more suitable for industrial fermentation may be used for the controlled digestion of plant wastes for the production of chemical feedstocks [61, 140, 150].

Mixed rumen organisms have been used as starter inocula for digesters fermenting agricultural wastes such as manure. The climax population in fermentation systems varies considerably from the inoculum, but, in appropriately run fermenters, methane is a major product.

One of the most exploitable areas of studies of insect–micro-organism interactions is the use of entopathogenic bacteria, fungi and viruses as biocontrol agents against pests (see Chapter 4.2).

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